Bacterial adhesion to modified polyurethanes

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Summary

Adhesion and subsequent growth of bacteria to surfaces is a problem in many different areas like medicine, food industry, on ships and other industrial and environmental applications. Many of these applications dictate the use of non-toxic alternatives to prevent adhesion.

In **Chapter 1** the 'fouling triangle' is presented, consisting of the most important physicochemical properties that determine adhesion of microorganisms to surfaces: hydrophobicity, elasticity and rugosity. The **aim** of this thesis was to verify this triangle by testing modified polyurethane coatings with varying hydrophobicity and elasticicty. It should be realised, however, that many other factors are relevant in adhesion as well, like bacterial variety and variability and conditioning film formation, which are addressed in this thesis as well.

In many situations of bacterial deposition, it is difficult to determine the exact mass transport conditions, particularly in the marine environment and therefore we investigated the influence of the type of flow chamber on the adhesion results under different mass transport conditions in **Chapter 2**. Deposition onto glass in a parallel plate (PPFC) and in a stagnation point flow chamber (SPFC) of *Marinobacter hydrocarbonoclasticus*, *Psychrobacter* sp., and *Halomonas pacifica*, suspended in artificial seawater, was compared in order to determine the influence of methodology on bacterial adhesion mechanisms. The three strains had different cell surface hydrophobicities, with water contact angles on bacterial lawns ranging from 18 to 85 degrees. Bacterial zeta potentials in artificial seawater were essentially zero, and because of the high ionic strength electrostatic interactions were of minor importance. The three strains showed different adhesion kinetics and the hydrophilic bacterium *H. pacifica* had the greatest affinity for hydrophilic glass. On average, initial deposition rates were two to three fold higher in the SPFC than in the PPFC, possibly due to the convective fluid flow toward the substratum surface in the SPFC causing more intimate contact between a substratum and a bacterial cell surface than the gentle collisions in the PPFC. The ratios between the experimental deposition rates and theoretically calculated deposition rates based on mass transport equations, differed not only per strain, but were also different for both flow chambers indicating different mechanisms under both modes of mass transport. The efficiencies of deposition were higher in the SPFC than in the PPFC, 62 ± 4 and 114 ± 28 % respectively. Experiments in the SPFC were more reproducible than in the PPFC with standard deviations over triplicate runs of 8% in the SPFC and 23% in the PPFC. Likely, this is due to better-controlled convective mass

transport in the SPFC, as compared with the diffusion controlled mass transport in the PPFC. In conclusion, this chapter shows that bacterial adhesion mechanisms depend on the prevailing mass transport conditions in the experimental set-up used, which makes it essential in the design of experiments that a methodology is chosen with mass transport conditions resembling the problem investigated.

Flow chambers commonly used in the literature to study microbial adhesion to surfaces under environmentally relevant hydrodynamic conditions were analyzed on a more theoretical basis in **Chapter 3a**. Four different PPFC's were analyzed in order to determine whether the expected hydrodynamic conditions, controlling both mass transport and detachment forces, were actually achieved. Furthermore, the different PPFC's were critically evaluated based on the size of the area where the velocity profile is established and constant under a range of flow rates, indicating that valid observations can be made. Velocity profiles in the different chambers were calculated using a numerical simulation model based on the finite element method and found to coincide with those measured with particle image velocimetry. Environmentally relevant shear rates between 0 and 10000 s^{-1} could only be measured over a sizeable proportion of the substratum surface for 2 out of the 4 PPFC's. Two models appeared flawed in the design of their in- and outlet and only allowed the development of a stable velocity profile for shear rates up to 0.5 and 500 s^{-1}, respectively. For these PPFC's the in- and outlet were curved and modeled shear rates deviated from calculated shear rates up to 75%. It was concluded, that PPFC's used for the study of microbial adhesion to surfaces should be designed to have their in- and outlet in line with the flow channel. Alternatively, the channel length should be increased to allow more length for the establishment of the desired hydrodynamic conditions. In C**hapter 3b** the velocity profile of fluid flow in the SPFC used in this thesis for deposition of bacteria was reported, together with the advantages and disadvantages of the SPFC. In a laminar flow regime (Reynolds number lower than 1400), shear rates increasing from the stagnation point where shear was 0 s^{-1}, up to 7000 s^{-1} were reached.

The formation of a conditioning film of organic compounds adsorbed from seawater, affects the properties of substratum surfaces prior to bacterial adhesion. **Chapter 4** describes deposition of the marine bacterial strains on glass, which was conditioned prior to bacterial adhesion for one hour with natural seawater. The seawater was collected in different seasons in order to determine the effect of seawater composition on the conditioning film and bacterial adhesion to it. The presence of a conditioning film was demonstrated by an increase in water contact angle from 15 degrees on bare glass to 50 degrees on the conditioned glass, concurrent with an increase in the amount of adsorbed organic carbon and nitrogen, as measured by X-ray photoelectron spectroscopy. Multiple linear regression analysis on initial deposition rates with as explanatory variables the temperature, salinity, pH and concentration of dissolved organic carbon (DOC) of the seawater at the time of collection, showed that the concentration of DOC was most strongly associated with the initial deposition rates of the three strains. Initial deposition rates of the two most hydrophilic strains to a conditioning film, increased with the concentration of DOC in the seawater, whereas the initial deposition rate of the most hydrophobic strain decreases with an increasing concentration of DOC.

Deposition of the marine bacterial strains with different cell surface hydrophobicities from artificial seawater to modified polyurethane coatings on glass with different surface tensions and elastic moduli was studied in situ in a PPFC and SPFC in **Chapter 5**. Different surface tensions of the coatings were established by changing the amount of fluorine, whereas using more or less branched polymers made different elastic moduli. Surface tensions of the coatings, derived from measured contact angles with liquids, ranged from 11.9 to 44.9 mJ m^{-2}, while the elastic moduli, derived from force-distance curves as measured with an atomic force microscope were between 1.5 and 2.2 GPa. In both flow chambers, the most hydrophilic bacterium *H. pacifica* adhered preferentially to the more hydrophilic, non-fluoridated coating, whereas the most hydrophobic bacterium *M. hydrocarbonoclasticus* showed a greater preference for the more hydrophobic coating. Bacterial adhesion in the PPFC was not influenced by the elastic modulus of the coatings, but in the SPFC bacteria adhered in higher numbers to hard surfaces than to coatings of lower elastic moduli.

The impact of conditioning films adsorbed from natural seawater to four polyurethane coatings with different hydrophobicity, elasticity and roughness was studied for three different marine bacterial strains in a multiple linear regression analysis in **Chapter 6**. The water contact angle on hydrophobic coatings decreased on average 8 degrees and increased on average by the same amount on hydrophilic coatings. These changes were accompanied by increased concentrations of oxygen and nitrogen on the surface as determined with XPS, indicative for adsorption of proteinaceous material. Furthermore, the mean surface roughness increased on average 4 nm after conditioning film formation. Many studies had shown relationships between substratum hydrophobicity, charge or roughness with bacterial adhesion, although it was obvious that bacterial adhesion is governed by an interplay of different physicochemical properties and multiple regression analysis would be more suitable to reveal mechanisms of bacterial adhesion. Multiple linear regression analysis revealed that changes in deposition due to conditioning film formation of *M. hydrocarbonoclasticus*, *Psychrobacter* sp. SW5H and *H. pacifica* in a SPFC, could be explained in a model comprising hydrophobicity and the prevalence of nitrogen rich components on the surface for the most hydrophobic strain. For the two more hydrophilic strains, deposition was governed by a combination of surface roughness and hydrophobicity. Elasticity was not a factor in bacterial deposition to conditioning films.

Bacterial adhesion occurs in many different environments and often causes problems. In **Chapter 7**, bacterial deposition to modified polyurethanes was compared in the marine and medical environment. Various mechanisms appeared to be operative in bacterial adhesion to the ployurethanes. Therefore it could be questioned, whether bacterial adhesion to surfaces can ever be captured in one generally valid mechanism, especially since the majority of studies on bacterial adhesion comprised two strains or less. Based on studies performed here using surface free energy and elasticity, it was clear that the nature of the experimental strains and surfaces can lead to different conclusions and bacterial deposition can therefore hardly be generalized.

In the general discussion (**Chapter 8**), it is stated that physicochemical properties of surfaces play an important role in bacterial adhesion and in the development of non-stick coatings, but the great variety in bacteria present in any environment system will always yield selection of strains are able to adhere. The adhered bacteria cause problems by secreting toxins and may promote subsequent adhesion of other organisms. A combination of different innovative strategies can be successful to avoid fouling. Physicochemical properties can be used to diminish or weaken bacterial adhesion to surfaces and thereby facilitate ‘cleansing’. An improved term for non-stick coatings would therefore be ‘easy-to-clean coatings’. The polyurethanes can thus be of great help in combination with cleansing strategies like moments of high shear, mechanical cleaning or more advanced strategies like removal of bacteria by electric fields, ultrasound, or passage of air bubbles.